by leaning them against a length of glass or metal rod 6 or 7 mm in diameter. (*Figure 14.1*).

When pouring plates raise the lid only far enough to permit the mouth of the tube or bottle to enter. Pour about 12–15 ml in each plate. Dry plates slightly open (*Figure 14.2*) in an incubator, and store medium side up in a refrigerator.

Figure 14.2. Drying a plate

Testing Culture Media. 'Efficiency of Plating' (EOP)

New batches of culture media, particularly media like DCA or Wilson and Blair, may vary considerably and should be tested in the following way.

Prepare serial tenfold dilutions, for example, 10^{-2} to 10^{-7} of cultures of various organisms which will grow on or be inhibited by the medium. For example, when testing DCA use *Sh.sonnei*, *S.typhi*, *S.typhimurium*, several other salmonellae and *Esch.coli*. Use

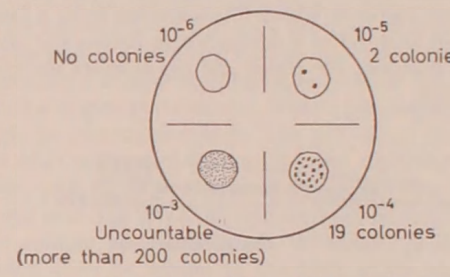

Figure 14.3. Miles and Misra count

at least four well-dried plates of the test medium for each organism and at least two plates each of a known satisfactory control medium, and of a general medium, e.g. MacConkey or Lemco agar. Do Miles and Misra drop counts with the serial dilutions of the organisms on these plates so that each plate is used for several dilutions. (*Figure 14.3*) (See p. 180.)

Count the colonies, tabulate the results and compare the performances of the various media. These may suggest that in a new

To 1000 ml of peptone water add 10 g of the appropriate fermentable substance and one of these indicators:

(*a*) 1 per cent Andrade indicator (pH 5–8)
(*b*) 5 per cent of a 0·2 per cent phenol red solution (pH 6·8–8·4)
(*c*) 1–2 per cent of a 0·2 per cent bromthymol blue solution (pH 6·0–7·6)
(*d*) 1–2 per cent of a 0·4 per cent bromcresol purple (pH 5·2–6·8)

Sterilize by steaming for 20 min on each of 3 successive days. Alternatively, bottle the peptone water plus indicator in 100 ml lots and autoclave. For use add sterile (filtered) solution of the carbohydrate to give a final concentration of 1 per cent and distribute aseptically into previously sterilized tubes.

Robinson's Serum Water Sugars

Some organisms will not grown in peptone water sugars. Robinson's serum water medium is superior to that of Hiss.

Peptone	5 g
Disodium hydrogen phosphate	1 g
Water	1000 ml

Steam for 15 min, adjust to pH 7·4 and add 250 ml horse serum. Steam for 20 min, add 10 ml Andrade indicator and 1 per cent of appropriate sugar (0·4 per cent starch).

Unheated serum contains diastase, and may contain a small amount of fermentable carbohydrate so a buffer is desirable, particularly in starch fermentation tests.

Sugar Media for Other Purposes

For *Neisseria*, which do not like a liquid medium, add 1 per cent agar to peptone water and 5 per cent of 0·2 per cent phenol red. Autoclave at 115°C for 10 min. To 100 ml add 10 ml of a 10 per cent solution of the appropriate sugar and 10 ml of rabbit serum. Tube and slope

For the sugar reactions of staphylococci and micrococci use Baird-Parker[8] formula.

Yeast extract	1 g
$NH_4H_2PO_4$	1 g
KCl	0·2 g
$MgSO_4.7H_2O$	0·2 g
Agar	12 g
Water	1000 ml